Morphological and genetic variations of *Diplodus vulgaris* along the Tunisian coasts

by

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Abstract. – In order to characterize populations of *Diplodus vulgaris* (Geoffroy St.-Hilaire, 1817) along the Tunisian coasts and to test the prospective role of geographical barrier of the Siculo-Tunisian Strait, we performed a study of morphological and genetic variability of samples collected in six sites distributed on each side of the Strait. Two types of morphological descriptors, 25 truss elements and six complementary conventional linear measurements, and genetic markers (allozymes of four enzyme systems) were used. Multivariate analyses revealed morphological variations among the prospected localities. El Biban sample differs by the suborbital part of the head and the eye diameter, while those of Ghar El Melh and Mahdia diverge mainly by the length and height of the head. Compared to all remaining samples, El Biban specimens showed a large head. The study of genetic polymorphism showed the absence of any genetic structuring among *Diplodus vulgaris* and the presence of an homogeneous (global FST = 0.004) panmictic unit (f = 0.046 NS). Thus, the Siculo-Tunisian Strait does not seem to act as a barrier between the populations, at least at the scale of the Tunisian coast. The morphological diversity observed seems to reflect the environmental heterogeneity and is not structured by the Siculo-Tunisian Strait (phenotypic plasticity).

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Résumé. – Variations morphologique et génétique de *Diplodus vulgaris* le long des côtes tunisiennes.

Pour caractériser les populations de *Diplodus vulgaris* (Geoffroy St.-Hilaire, 1817) le long des côtes tunisiennes et tester le rôle éventuel de barrière géographique du détroit siculo-tunisien, nous avons étudié la variabilité morphologique et génétique d'échantillons collectés dans six sites de part et d'autre du détroit. Deux types de descripteurs morphologiques, 25 éléments de truss et six mesures linéaires conventionnelles complémentaires, et des marqueurs génétiques (allozymes de quatre systèmes enzymatiques) ont été utilisés. Des analyses multivariées ont révélé des variations morphologiques entre les localités prospectées. Ainsi, l'échantillon d'El Biban diffère par la partie suborbitale de la tête et le diamètre de l'œil alors que ceux de Ghar El Melh et de Mahdia divergent principalement par la longueur et la hauteur de la tête. Par rapport à l'ensemble des échantillons, les spécimens d'El Biban ont une plus grosse tête. L'étude du polymorphisme génétique a montré l'absence de toute structuration génétique chez *Diplodus vulgaris* et la présence d'une unité panmictique ($f = 0.046$ NS) homogène ($F_{\text{ST global}} = 0.004$). Ainsi, le détroit siculo-tunisien ne semble pas jouer le rôle de barrière entre les populations, au moins à l'échelle de la côte tunisienne. La diversité morphologique (plasticité phénotypique) semble refléter l'hétérogénéité environnementale et n'est pas structurée par le détroit siculo-tunisien.

Key words
Sparidae
Diplodus vulgaris
Tunisian coasts
Morphological variation
Genetic homogeneity
Allozymes

Marine habitats are dispersive systems, which do not exhibit clear physical barriers, favouring gene exchange among populations (Palumbi, 1994) and contributing to limit the action of *genetic differentiation factors among populations* (Turan *et al*., 2004). However, some geographic marine areas are recognized to play the role of transition zone for several marine species (Borsa *et al*., 1997; Bargelloni et *al*., 2005; Patarnello *et al*., 2007; Galarza *et al*., 2009; Mejri *et al*., 2012). In the Mediterranean Sea, the Siculo-Tunisian Strait (STS), located between Cap Bon in Tunisia and Mazara del Vallo in Italy, constitutes a physical boundary between the eastern and western Mediterranean basins (Béranger *et al*., 2004). It seems to constitute a geographic barrier for some marine fish species, which are subject to genetic transition in this area (Bahri-Sfar *et al*., 2000; Stefanni and Thorley, 2003; Zardoya *et al*., 2004; Mejri *et al*., 2009, 2011). Several genetic studies, especially on sparids, were carried out along this area, highlighting different genetic patterns (Ben Slimen *et al*., 2004; Hammami *et al*., 2007; Fassatoui *et al*., 2009; Kaouèche *et al*., 2011). The transition between the two basins seems to be perceived in different ways depending on the species since a strong to moderate genetic divergence is observed in some species, whereas others show homogenous pattern. For instance, for *Lithognathus mormyrus*, no evidence of genetic structuring was observed (Hammami *et al*., 2007). In contrast, a strong differentiation was detected among Tunisian *Sparus aurata* between groups located in the eastern and western Mediterranean basins (Ben Slimen *et al*., 2004), whereas a slight differentiation was observed between distant samples of

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Pagellus erythrinus (Fassatoui *et al*., 2009). Finally, in the case of *Diplodus sargus*, a differentiation was revealed by allozymes and was attributed to small-scale, not patterned, genetic heterogeneity among local populations, whereas the mtDNA data indicate a genetic homogeneity of the white seabream populations among the same localities across the STS (Kaouèche *et al*., 2011).

In addition, a sufficient degree of isolation may result in notable morphological differentiation among stocks within a species (Pepin and Carr, 1993). Beside the primary interest in understanding the patterning of fish populations, the description of the morphological variation between populations and the identification of correlated degree of genetic pattern help to implement the appropriate management strategies of Mediterranean fisheries. In this context, morphometric characters (Turan and Basusta, 2001; Palma and Andrade, 2002; Turan, 2004; Bahri-Sfar and Ben Hassine, 2009; Hammami *et al*., 2011) or morphometric characters associated to genetic data (Cabral *et al*., 2003; Mejri *et al*., 2012) have been commonly used as markers to characterize and delimit geographic stocks (Arculeo *et al*., 2003).

The two-banded seabream, *Diplodus vulgaris* is one of the most frequent and abundant sparid in the Mediterranean Sea and along the eastern Atlantic coast (Bauchot and Hureau, 1986). The species is generally found associated with both rocky and sandy bottoms. Juveniles inhabit coastal lagoons and estuaries (Macpherson, 1998). *D. vulgaris* life cycle is characterized by an offshore pelagic larval dispersion during 29-58 days followed by a nectobenthic adult phase. These characteristics give great dispersal potential, since in marine environments, early-life-history traits and dispersal capabilities may influence exchanges of gene flow between populations (Palumbi, 2004; Galarza et *al*., 2009).

In this context, we have undertaken the characterization of Mediterranean populations of two-banded seabream across the STS. Effectively, despite the commercial importance of this species, studies are scarce. In this regard, a first genetic study of Mediterranean *Diplodus vulgaris* populations, using allozyme markers in seven samples from Tyrrhenian, Aegean, Ionian and Adriatic Seas and also both sides of the STS, demonstrated a distinction of the Adriatic Sea sample only (Arculeo *et al*., 2003). However, Galarza *et al.* (2009) have shown a genetic transition across the Almeria-Oran front using microsatellite markers, but with sampling that did not cover the entire distribution area of *D. vulgaris* in the Mediterranean Sea. In the present study, we aimed to check whether morphological variation in *D. vulgaris* was in relation with the STS, then we examined the possible correlation with genetics and ecological features of the studied populations. For that purpose, we collected samples along the Tunisian coasts, on both sides of the STS in order to characterize the population of *D. vulgaris* in this transition area with heterogeneous environments. The morphometric study was based on body shape descriptors (conventional linear and truss measurements), while genetic investigation was based on allozyme polymorphism.

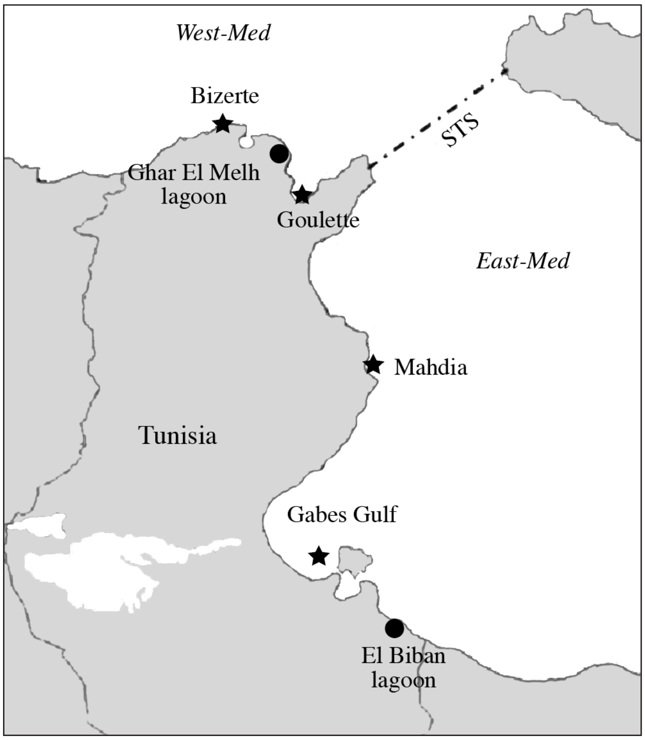

Figure 1. - Locations of sampling sites along the Tunisian coasts. ★: marine samples. ●: lagoon samples. STS: Siculo-Tunisian Strait. West-Med: Western Mediterranean basin. East-Med: Eastern Mediterranean basin.

MATERIAL & METHODS

Sampling

Samples were collected from six study sites (4 marine and 2 lagoonal), distributed on each side of the STS and covering northeastern (Bizerta Bay, Ghar El Melh lagoon and Goulette) and southeastern (Mahdia, Gabes Gulf, and El Biban Lagoon) sectors of Tunisian coastline (Fig. 1). These localities differ by their environmental features (Tab. I). Sample size ranged from 27 to 51 individuals (Tab. I). All fishes were adults. They were captured using trammel nets during the period between September 2007 and December 2008. The samples were brought to the laboratory in order to take the defined morphometric measurements, dissect and remove pieces of muscle and liver, which we stored at –20°C for allozyme exams.

Morphometric data

In order to describe the shape of collected fish, the truss approach was used to create a network describing fish body outline (Strauss and Bookstein, 1982). This approach is

Table I. - Sample locations of specimens of *D. vulgaris* and main environmental features of study sites. MSL: mean standard length.

Geographic location	North of the Siculo-Tunisian Strait			South of the Siculo-Tunisian Strait		
	Bizerte Bay	Goulette	Ghar El Melh lagoon	Mahdia	Gabes Gulf	El Biban lagoon
Sites code	MBIZV	MGOUV	LGEMV	MMAHV	MGABV	LBIBV
Sample size	48	33	27	35	51	37
MSL (cm)	12.069 ± 1.568	11.401 ± 1.16	10.040 ± 0.877	10.798 ± 2.038	10.016 ± 1.702	14.075 ± 0.735
Range of standard lengths	[10.25: 15.25]	[10.28: 13.85]	[9.82:12.25]	[9.58:12.81]	[10.29:14.02]	[11.95:15.47]
Environment type	marine	marine	lagoon	marine	marine	lagoon
Geographic coordinates	37°16'60"N; 9°58'0"E	36°47'43"N; 10°16'43"E	37°10'N; 10°11'E	35°30'N; 11°04' E	34°04'48"N; 10°28'36"E	33°16'N; 11°17"E
Bottom types	Rocky (Oueslati, 1993)	Muddy-sandy Ben Alaya (1972)	Muddy (Moussa *et al*., 2005)	Rocky (Ben Hassine *et al*., 1999)	Soft, sandy and muddy (Burollet, 1979)	Sandy (Medhioub *et al*., 1986)
Vegetation	*Posidonia* *Caulerpa* *Jania rubens* Molinier and Picard (1954)	*Cymodocea* *Caulerpa* *Posidonia* Azouz (1973)	*Ruppia cirrhosa* *Cladophora* sp. (Algae are the most dominants) Moussa *et al*. (2005)	Phanerogams and Algae Ben Hassine *et al*. (1999)	*Cymodocea* *Posidonia* *Caulerpa* Ben Othman (1971)	*Zostera noltii* *Cymodocea nodosa* *Caulerpa prolifera* *Lithothamnium* sp. (Phanerogams are the most dominants) Guélorget *et al*. (1982)
Tide	0.40 m Blanco (1992)	0.45 m Ben Salem (2003)	0.08-0.22 m Moussa *et al*. (2005)	≈ 20 cm	2 m Mensching (1968)	± 0.5 m (max ≈ 1 m) Lemoalle and Vidy (1984)
Currentology	Mediterranean Atlantic water Astraldi *et al*. (1999)	Mediterranean Atlantic water Astraldi *et al*. (1999)	Mediterranean Atlantic water Astraldi *et al*. (1999)	Levantine intermediate water Astraldi *et al*. (1999)	Levantine intermediate water Astraldi *et al*. (1999)	Levantine intermediate water Astraldi *et al*. (1999)
Temperature	21-22°C (low depths) 15-16°C (deep) Oueslati (1993)	13°C-28°C Daly-Yahia (1998)	10.3 °C-29.7°C Ben Hassine (1983)	15°-25°C Zakham *et al*. (2005)	≈ 21.45°C Drira (2009)	13°C-30°C Neifar (2001)
Turbidity	low turbidity Boufahja et *al*. (2010)	high turbidity Pergent and Kempf (1993)	high turbidity Moussa *et al*. (2005)	low turbidity Pergent and Kempf (1993)	high turbidity Drira (2009)	low turbidity Lemoalle (1986)
Salinity	≈ 37‰ Oueslati (1993)	36.5- 37‰ (low depths) 37-38‰ (deep) Daly-Yahia (1998)	36‰-51‰ Moussa *et al*. (2005)	≈ 37.1 % Alioua and Harzallah (2008)	38-39 ‰ Hamza (2003)	45‰-50‰ Neifar (2001)

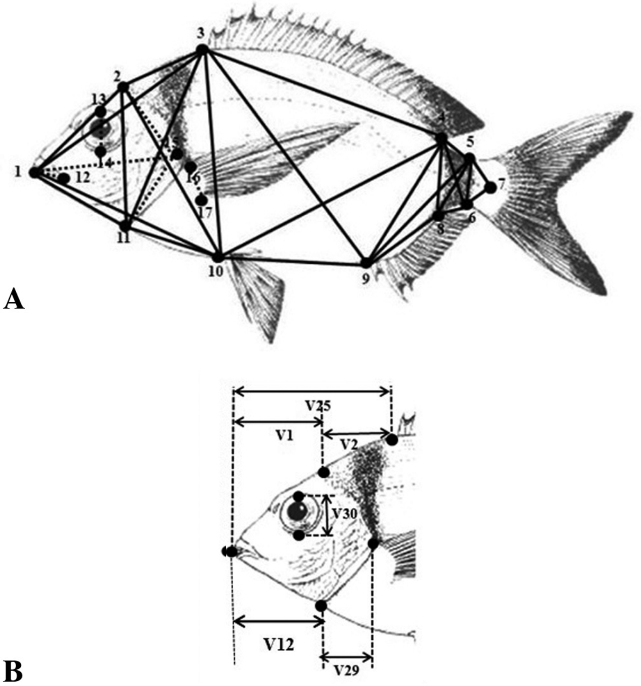

Figure 2. - **A**: Location of the 11 landmarks (1-11) used for constructing the truss network on *D. vulgaris* and the six additional points (12-17) used to draw the conventional linear measurements. Landmarks and additional points illustrated as black dots. Truss network illustrated as continuous lines. Conventional linear measurements illustrated as discontinuous lines. **B**: Discriminated head region and discrimination related variables.

optimal for extracting body shape differences among populations. The left side of each fish was photographed with a high-resolution digital camera mounted on a tripod. Morphological landmarks were selected around the outline of the fish form (Fig. 2). Landmark coordinates were performed on digital images using Visilog 6.480 software. Eleven landmarks determining 25 measurements were recorded (Fig. 2). Six points were added to obtain complementary conventional linear measurements, i.e. eye diameter, upper and lower head height, snout length, operculum length, and width of the pectoral fin base (Fig. 2; Annex I). Thus, 31 measurements were analysed (Tab. I). In order to eliminate any size effect, size-dependent variation of morphometric measurements was removed using an allometric transformation (Reist, 1985): $M_{\text{trans}} = \log M - \beta (\log \text{SL} - \log \text{SL}_{\text{mean}})$ where M_{trans} is the transformed measurement, M the original measurement, β the within-group slope regressions of log M against log SL, SL the standard length of the fish and SL_{mean} the overall mean of the standard length.

The analysis of variance (ANOVA) was performed to test the statistical significance of mean variations among samples and for each measurement. Multivariate analyses were used to elucidate pattern of the morphological variation between samples. The t-test was established to infer whether the averages of each variable are significantly different between two considered samples.

The discriminant function analysis (DFA) was assessed in order to carry out the degree of similarity between the studied samples and the relative importance of each measurement for group separation. Wilks' λ values were estimated to test the significance of observed discrimination for a combination of variables. The classification success rate (PCS) was evaluated based on the percentage of individuals correctly assigned into the original sample. Statistical analyses were performed using R 2.11.1 software.

Allozyme data

Protein extraction was released in Tris buffer (pH = 6.8). Electrophoresis and specific staining were performed according to Pasteur *et al.* (1987). Four enzymatic systems, showing clear zymograms, were used in this analysis: alcohol deshydrogenase EC 1.1.1.1 (ADH; TC 8), isocitrate deshydrogenase EC 1.1.1.4.2 (ICD; TC 8), lactate deshydrogenase EC 1.1.1.27 (LDH; TC 8), glucose phosphomutase EC 5.4.2.1 (PGM; TC 8) and glucose phosphate isomerase EC 5.3.1.9 (PGI; TC8). Eight polymorphic loci were scored (ADH^*, $ICD\text{-}1^*$, $ICD\text{-}2^*$, LDH^*, $PGI\text{-}1^*$, $PGI\text{-}2^*$, $PGI\text{-}3^*$ and PGM^*). Nomenclature of loci and alleles was written according to Shaklee *et al.* (1990).

Genetic diversity was described through estimating the unbiased expected heterozygosities (H_e) (Nei and Chesser, 1983), mean number of alleles per locus (A_m), and number of exclusive alleles (A_e). Departure from Hardy-Weinberg Equilibrium (HWE) was tested with the estimator f of Wright fixation index (F_{IS}) (Wright, 1969) according to Weir and Cockerham (1984). The estimator θ (Weir and Cockerham, 1984) of F_{ST} was calculated between populations in order to estimate the genetic differentiation between samples. All analyses were carried out using Genetix v.4.05 software (Belkhir *et al.*, 2004).

RESULTS

Morphometric analysis

The univariate analysis (ANOVA) shows significant differences ($P < 0.001$) among localities for all studied variables (Tab. I). The projection of the six samples on the plan defined by DF1 and DF2 illustrates the variation among samples (Fig. 3). More precisely: the first function DF1, which explains 28% of the global variation, highlights the distinction of the southern lagoon sample (El Biban lagoon), whereas the second function (DF2), which explains 24% of the global variation, allows the discrimination of the northern lagoon (Ghar El Melh lagoon) and an eastern marine sample, namely Mahdia; the remaining three localities are undifferentiated according to DF1 and DF2

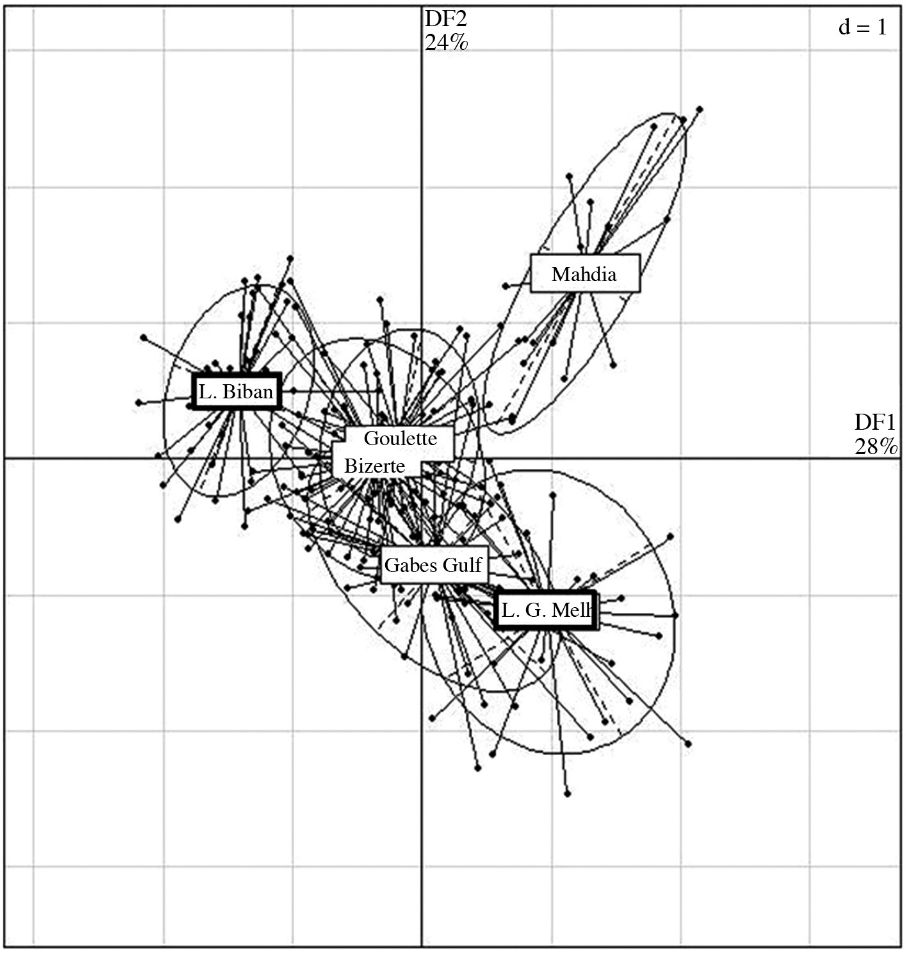

Figure 3. - DFA scores of morphometric characters using conventional linear measurements and truss elements on the plan DF1-DF2.

(Fig. 3). The discrimination of El Biban lagoon is essentially highlighted by two truss measurements located in the head region and the eye diameter (V1, V2, V30; Fig. 2; Annex I). In fact, the head of El Biban lagoon specimens has larger eyes and a longer predorsal edge than all other samples. The application of t-test among these three discriminative measurements showed significant values (Tab. II). El Biban lagoon sample has the highest means of the truss variables ($V2_{Biban}$ = 2.261 mm; $V25_{Biban}$ = 6.984 mm) and the eye diameter ($V30_{Biban}$ = 1.274 mm) than Ghar El Melh lagoon ($V2_{GharM\,=}$ 1.319 mm, $V25_{GharM\,=}$ 4.697 mm and $V30_{GharM\,=}$ 0.828 mm) and Mahdia ($V2_{Mahdia\,=}$ 1.054 mm, $V25_{Mahdia\,=}$ 4.837 mm and $V30_{Mahdia\,=}$ 1.100 mm).

Ghar El Melh lagoon sample and Mahdia marine sample are discriminated along the second function DF2, defined by three measurements related to the head dimensions (Fig. 2: V1, V12, V29; Tab. I). Significant Average differences between Ghar El Melh and Mahdia are highlighted by V1 and V29 (Tab. II) with the lowest average for the front head length (Fig. 2: V1) in Ghar El Melh population. In addition, pairwise significant average comparisons show that in all cases, El Biban lagoon fish have the biggest head (Tab. II). Observed morphometrical distinction among Tunisian *D. vulgaris* population was confirmed by a significant value of Wilks' λ criterion (Wilks $\lambda = 0.0236$; $F = 7.084$; $P < 0.001$).

Finally, the overall assignment of individuals into their original sample (PCS) was 82.16%. The percentage values of specimens correctly classified into the original groups were ranged from 61% to 100% (Tab. III), the highest percentage being observed in El Biban and Mahdia samples. This classification confirmed the clear discrimination of these samples (Fig. 3).

Allozymes

Twenty-one alleles were observed among the eight typed polymorphic loci. Four unique alleles were detected (Tab. IV). Three of them are observed in the southern coasts (*ADH** 120 in El Biban lagoon; *PGI-2** 120 and *PGI-3** 120 in Gabes Gulf). A fourth one is detected in Ghar El Melh lagoon (*PGI-3** 80). However, the all four are considered as rare alleles. Allele frequencies ranged from 0.019 for *PGI-3** 80 to 0.027 for *ADH** 120. Diversity parameters showed that the highest values of average number of alleles ($A_m = 2.125$) and average expected heterozygosity ($He = 0.072 \pm 0.014$)

are observed in the sample from the Gabes Gulf (Tab. IV).

Global F_{IS} value estimated for all samples is low and not significant ($f = 0.046$). Multilocus f values in each locality are not significant (Tab. IV). They range from -0.024 ^{NS} in El Biban lagoon sample to 0.209 ^{NS} in Goulette sample.

Global F_{ST} value is also low and not significant (0.004), showing the absence of any differentiation among studied samples. The estimation of pairwise θ values confirms this homogeneity. Pairwise θ values are not significant and range from 0.000 to 0.013 between the Goulette sample, located in the north Tunisian coast, and the El Biban lagoon sample which is a southern sample (Tab. V, Fig. 1).

The estimation of pairwise genetic distances between the samples reveal low values (Tab. V), improving obtained results with θ estimator.

Table II. - Average comparisons using Student's t-test; ddl: degree of liberty. **A**: t-test values calculated between discriminated variable averages for distinctness samples along DF1. **B**: t-test values calculated between discriminated variable averages for distinctness samples along DF2. See table I for sample site codes.

A	ddl	V2	V25	V30
LBIBV/MBIZV	84	16.118***	9.098***	45.728***
LBIBV/LGEMV	63	65.527***	43.627***	141.47***
LBIBV/MGOUV	69	15.374***	8.452***	87.418***
LBIBV/MMAHV	71	92.277***	26.923***	29.823***
LBIBV/MGABV	87	31.302***	19.283***	76.911***

B		Student's t-test		
	ddl	V1	V12	V29
MMAHV/MBIZV	82	-4.595***	-6.226***	-16.551***
MMAHV/LGEMV	61	-4.566*	ns	-7.817*
MMAHV/MGOUV	67	ns	ns	-14.329**
MMAHV/MGABV	85	8.174***	4.726**	9.115*
MMAHV/LBIBV	71	-14.577***	-18.216***	-58.691***
LGEMV/MBIZV	74	-9.571*	-10.129***	-21.524***
LGEMV/MGOUV	59	ns	-4.994**	-21.014***
LGEMV/MGABV	77	ns	ns	ns
LGEMV/LBIBV	63	-23.68***	-34.769***	-68.666***

DISCUSSION

The analyses of morphometric data of *Diplodus vulgaris* collected in marine and lagoonal environments along the Tunisian coasts on both sides of the STS revealed morphological variation among localities but do not show a relation with the presence of the STS. The lagoon sample at Ghar El Melh and at El Biban and the southeast marine sample at Mahdia exhibit each distinguishing head morphology, whereas all other marine samples exhibit close median morphology of the head (Bizerta, Goulette and Gabes Gulf). The specimens from El Biban lagoon are notably characterized by their biggest head when compared to other samples.

Morphological differences in the head region have been documented in several marine fish species (Turan, 2004; Wainwright *et al.*, 2004; Mejri *et al.*, 2012) and similar results were obtained in other sparid species. For instance, a significant degree of morphological dissimilarities was evidenced between sparid populations of *Diplodus sargus*, *D. puntazzo* from four Mediterranean (Spain, Italy and Greece) and Atlantic (Portugal) localities, among which more than

Table III. - Percentage of specimens classified in each group.

	El Biban lagoon	Ghar El Melh lagoon	Bizerte Bay	Gabès Gulf	Goulette	Mahdia
El Biban lagoon	100	–	–	–	–	–
Ghar El Melh lagoon	–	82	4	7	7	–
Bizerte Bay	10	2	70	6	10	2
Gabès Gulf	6	–	14	80	–	–
Goulette	3	–	34	3	61	–
Mahdia	–	–	–	–	–	100

Table IV. - Genetic diversity parameters in *D. vulgaris* samples from the Tunisian coasts: sample size (N), number of exclusive alleles (A_e), mean number of alleles per locus and per population (A_m), average unbiased expected heterozygosity (H_e), Weir and Cockerham (1984) multilocus fixation index (f) measuring departure from theoretical Hardy-Weinberg equilibrium, confidence interval (±), non significant (NS).

	Bizerte Bay	Goulette	Ghar El Melh lagoon	Mahdia	Gabès Gulf	El Biban lagoon
N	48	33	27	35	51	37
Ae	–	–	1	–	2	1
Am	2	1.625	2	1.5	2.125	1.625
He	0.062 ± 0.014	0.056 ± 0.022	0.066 ± 0.016	0.030 ± 0.013	0.072 ± 0.014	0.035 ± 0.013
f	-0.047NS	0.209 NS	0.112 NS	-0.030 NS	0.091 NS	-0.024 NS

Table V. - Genetic distance values (Nei, 1987) (above diagonal) and θ values (below diagonal), estimator of F_{ST} according to Weir and Cockerham (1984) among *D. vulgaris* samples for allozymic data.

	Mahdia	Bizerte Bay	Goulette	Ghar El Melh lagoon	El Biban lagoon	Gabès Gulf
F_{ST}	0	0	0	0	0	0
Mahdia		0.000	-0.000	0.000	0.000	0.000
Bizerte Bay	0.001NS		0.000	0.000	0.000	0.001
Goulette	0.000 NS	0.000 NS		0.000	0.001	0.001
Ghar El Melh lagoon	0.011 NS	0.006 NS	0.002 NS		-0.000	0.000
El Biban lagoon	0.006 NS	0.002 NS	0.013 NS	0.003 NS		0.000
Gabès Gulf	0.005 NS	0.008 NS	0.010 NS	0.001 NS	0.007 NS	

50% were head-related (Palma and Andrade, 2002). Moreover, a morphological discrimination, assigned to the head length allows the distinction between lagoon populations of *Lithognathus mormyrus* (Hammami *et al.*, 2011, 2013). Finally, similar results regarding the head morphology were obtained by Sarà *et al.* (*1999*) on cultivated *D. puntazzo* reared under different conditions. So, if well documented, in the Mediterranean region, such morphological discrepancies in fish populations are attributed either to the impact of habitat differences (Palma and Andrade, 2002; Cabral *et al.*, 2003; Turan, 2004; Hammami *et al.*, 2011; Kaouèche, 2012), or to the interaction of both genetic and environmental components (Favaloro and Mazzola, 2006; Bahri-Sfar and Ben Hassine, 2009; Mejri et *al.*, 2012), with the rupture or reduction of gene flow attributed to the existence of fronts and straits acting as barriers (Bahri-Sfar et *al.*, 2000; Bargelloni et *al.*, 2005; Galarza et *al.*, 2009; Bahri-Sfar and Ben Hassine, 2009; Zittari-Chatti et *al.*, 2009; Mejri et *al.*, 2012). In this study, genetic analyses based on allozyme markers demonstrates the homogeneity among our samples. Therefore, we can consider that Tunisian *Diplodus vulgaris* constitute a homogeneous panmictic unit and that STS does not seem to act as a barrier to gene flow for this species at the scale of the Tunisian coast. This can be explained by a quite long pelagic larval duration of *D. vulgaris* and larval dispersal (29-58 days; Bauchot and Hureau, 1986), which favour gene flow and population homogenization. These results are not so surprising since low level of genetic structure was highlighted in the Mediterranean region for *D. vulgaris*, with only differentiation across the Almeria-Oran Front (Galarza *et al.*, 2009) and the discrimination of the Adriatic Sea population (Arculeo *et al.*, 2003). Moreover, a similar result (homogeneity) was observed with the related species *D. sargus* (Kaouèche *et al.*, 2011), which shares life history traits but displays a shorter phase of larval dispersal (21-30 days).

So, the morphological variations observed between the Tunisian two-banded seabream cannot be attributed to genetic differentiation. It is thus related to environmental differences between the studied sites and could reflect an adaptive divergence of populations.

The fact that the morphological variations concentrate in the head region leads us to search for eventual causes in fish diet (e.g. Walls *et al.*, 1993; Palma and Andrade, 2002; Hammami *et al.*, 2013). Indeed, *Diplodus vulgaris* is a demersal fish that feeds on benthic invertebrates (Fischer *et al.*, 1987). However, depending on environmental characteristics prevailing in each of these sites, the availability and abundance of potential prey vary from one site to another (Rosecchi, 1985). On the east coast of Tunisia, their preferential prey are crustaceans, molluscs, annelids and echinoderms, while secondary preys correspond to sponges and platyhelminths (Tekaya, 2009). In contrast, in the Gulf of Gabes, *D. vulgaris* feeds preferentially on bivalves and crustaceans, and secondarily on annelids and cephalopods (Bradai, 2000). This difference in diet composition may reflect the prey availability in relation to the heterogeneity of target environments. However, direct correlation between head shape of a population and its environment cannot be assumed. This is probably because of mainly multifactorial controls on prey availability. Indeed, precise information on the diet of the given populations (stomach contents along several seasons) is necessary to explain further head morphology.

So, since it is admitted that when a species colonizes a range of heterogeneous environments, a single phenotype is unlikely to be associated with high fitness throughout the range (Via *et al.*, 1995), a species must adapt to succeed, notably by being able to match phenotypes with local environments (Jørgensen *et al.*, 2008). Such adaptations may imply changes in morphology, reproduction or survival features (Stearns, 1983). Our study suggests that, on Tunisian coasts, the two-banded seabream is able to adapt to local environments through phenotypic plasticity alone, which is considered an optimal strategy to coping in heterogeneous environments (Jørgensen et *al.*, 2008). Conversely, the STS does not play any role in the patterning of the populations of *Diplodus vulgaris*, at least at the scale of the Tunisian coast.

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REFERENCES

ALIOUA M. & HARZALLAH A., 2008. - Imbrication d'un modèle de circulation des eaux près des côtes tunisiennes dans un modèle de circulation de la mer Méditerranée. *Bull. Inst. Natl. Sci. Tech. Mer Salammbô,* 35: 169-176.

ARCULEO M., LO BRUTTO S., SIRNA-TERRANOVA M., MAGGIO T., CANNIZZARO L. & PARRINELLO N., 2003. - The stock genetic structure of two Sparidae species, *Diplodus vulgaris* and *Lithognathus mormyrus*, in the Mediterranean Sea. *Fish. Res.,* 63: 339-347.

ASTRALDI M., BALOPOULOS S., CANDELA J., FONT J., GACIC M., GASPARINI G.P., MANCA B., THEOCHARIS A. & TINTORE J., 1999. - The role of straits and channels in understanding the characteristics of Mediterranean circulation. *Prog. Oceanogr.,* 44: 65-108.

AZOUZ A., 1973. - Les fonds chalutables de la région Nord de la Tunisie. 1. Cadre physique et biocoenoses benthiques. *Bull. Inst. Océanogr. Pêche Salammbô,* 2(4): 473-563.

BAHRI-SFAR L. & BEN HASSINE O.K., 2009. - Clinal variations of discriminative meristic characters of sea bass, *Dicentrarchus labrax* (Moronidae, Perciformes) populations on Tunisian coasts. *Cybium,* 33(3): 211-218.

BAHRI-SFAR L., LEMAIRE C., BEN HASSINE O.K. & BONHOMME F., 2000. -Fragmentation of sea bass populations in the western and eastern Mediterranean as revealed by microsatellite polymorphism. *Proc. R. Soc. Lond.,* B Biol., 267: 929-935.

BARGELLONI L., ALARCON J.A., ALVAREZ M.C., PENZO E, MAGOULAS A., PALMA J. & PATARNELLO T., 2005. - The Atlantic-Mediterranean transition: discordant genetic patterns in two seabream species, *Diplodus puntazzo* (Cetti) and *Diplodus sargus* (L). *Mol. Phylogenet. Evol*., 36: 523-535.

BAUCHOT M.L. & HUREAU J.C., 1986. - Sparidae. *In*: Fishes of the north-eastern Atlantic and the Mediterranean (Whitehead P.J.P., Bauchot M.L., Hureau J.C., Nielsen J. & Tortonese E., eds), pp. 883-907. Paris: Unesco.

BELKHIR K., BORSA P., GOUDET J., CHIKHI L. & BONHOMME F., 2004. - GENETIX V4.05 software sous Windows (TM) pour la génétique des populations. Laboratoire Génome et Populations. Université Montpellier II. Available at http://www.univ-montp2.fr/~genetix/ genetix/intro.htm.

BEN ALAYA H., 1972. - Répartition et condition d'installation de *Posidonia oceanica* Delile et *Cymodocea nodosa* Ascherson dans le golfe de Tunis. *Bull. Inst. Océanogr. Pêche (Salammbô)*, 2 (3): 331-416.

BEN HASSINE O.K., 1983. - Les Copépodes parasites de poissons Mugilidae en Méditerranée occidentale (côtes françaises et tunisiennes). Morphologie, Bio-écologie, cycles évolutifs. Thèse de Doctorat d'État, 452 p. U.S.T.L., Montpellier, France.

BEN HASSINE O.K., BENMANSOUR B., NEIFER L., BAHRI L., HAJI T., CHAOUACHI B., LABAIED M. & MAJED M., 1999. - L'état de la biodiversité dans les sites à activité halieutique du littoral Est de la Tunisie. *In*: L'homme et la mer, Tome I, pp. 277-333.

BEN OTHMAN S., 1971. - Observation hydrologiques : dragages et chalutages dans le Sud-Est tunisien. *Bull. Inst. Océanogr. Pêche Salammbô*, 2(2): 103-120.

BEN SALEM S., 2003. - Étude de l'environnement socio-économique de la zone côtière du Parc national de Zembra et Zemretta. Projet MedMPA, p. 69.

BEN SLIMEN H., GUERBEJ H., BEN OTHMEN A., OULD BRAHIM I., BLEL N., CHATTI N., EL ABED A. & SAID K., 2004. - Genetic differentiation between populations of gilthead seabream (*Sparus aurata*) along the Tunisian coast. *Cybium,* 28(1): 45-50.

BÉRANGER K., MORTIER L., GASPARINI G.P., GERVASIO L., ASTRALDI M. & CREPON M., 2004. **-** The dynamics of the Sicily Strait: a comprehensive study from observations and models. *Deep Sea Res. II,* 51: 411-440.

BLANCO B., 1992. - Protection du littoral du Gouvernorat de Bizerte. Conseil de l'ordre des ingénieurs; Comité régional de Bizerte, pp. 1-5.

BORSA P., NACIRI M., BAHRI L., CHIKHI L., GARCIA DE LEON F.J., KOTOULAS G. & BONHOMME F., 1997. - Intraspecific zoogeography of the Mediterranean: population genetic analysis on sixteen Atlanto-Mediterranean species (fish and invertebrates). *Vie Milieu,* 47: 295-305.

BOUFAHJA F., HEDFI A., AMORRI J., AÏSSA P., BEYREM H. & MAHMOUDI E., 2010. - Evaluation des réponses trophiques des nématodes libres de la baie de Bizerte (Tunisie) au stress pétrolier. *Bull. Inst. Sci., Rabat, Sect. Sci. Vie*, 32(2): 55-64.

BRADAI M.N., 2000. - Diversité du peuplement ichtyque et contribution à la connaissance des sparidés du golfe de Gabès. Thèse de Doctorat d'État, 600 p. Fac. Sci. Sfax, Tunisie.

CABRAL H.N., MARQUES J.F., REGO A.L., CATARINO A.I., FIGUEIREDO J. & GARCIA J., 2003. - Genetic and morphological variation of *Synaptura lusitanica* Capello, 1868, along the Portuguese coast. *J. Sea Res.,* 50: 167-175.

DALY-YAHIA M.N., 1998. **-** Dynamique saisonnière du zooplancton de la baie de Tunis (systématique, écologie numérique et biogéographie méditerranéenne). Thèse de Doctorat, 385 p. Fac. Sci. Tunis., Univ. Tunis II, Tunisie.

DRIRA Z., 2009. - Contribution à la compréhension du fonctionnement du golfe de Gabès : étude des caractéristiques dynamiques et structurales des communautés phytozooplanctoniques en relation avec la variabilité environnementale et les caractéristiques hydrographiques des zones côtières et océaniques. Thèse de Doctorat, 229 p. Fac. Sci., Univ. Sfax, Tunisie.

FASSATOUI C., MDELGI E. & ROMDHANE M.S., 2009. - A preliminary investigation of allozyme genetic variation and population structure in common pandora (*Pagellus erythrinus*, Sparidae) from Tunisian and Libyan coasts. *Ichthyol. Res.,* 56: 301-307

FAVALORO E. & MAZZOLA A., 2006. - Meristic character counts and incidence of skeletal anomalies in the wild *Diplodus puntazzo* (Cetti, 1777) of an area of the south-eastern Mediterranean Sea. *Fish. Phys. Biochem.,* 32: 159-166.

FISCHER W., BAUCHOT M.L. & SCHNEIDER M., 1987. - Sparidae. *In:* Fiches FAO d'identification des espèces pour les besoins de la pêche (révision 1). Méditerranée et Mer Noire. Zone de pêche 37, Vol. II (Vertébrés). Rome, FAO: 1343-1376.

GALARZA J.A., CARRERAS-CARBONELL J., MACPHERSON E., PASCUAL M., ROQUES S., TURNER G.F. & RICO C., 2009. - The influence of oceanographic fronts and early-life-history traits on connectivity among littoral fish species. *P.N.A.S.*, 106(5): 1473-1478.

GUÉLORGET O., FRISONI G.F. & PERTHUISOT J.P., 1982. - Contribution à l'étude biologique de la bhiret El Biban, lagune du Sud-Est tunisien. *Mém. Soc. Géol. Fr., N. S.*, 144: 173-186.

HAMMAMI I., BAHRI-SFAR L., KAOUÈCHE M. & BEN HASSINE O.K. 2007. - Genetic characterization of striped seabream (*Lithognathus mormyrus*) populations on both sides of a boundary area between eastern and western Mediterranean basins. *Cybium,* 31(2): 143-147.

HAMMAMI I., BAHRI-SFAR L. & BEN HASSINE O.K., 2011. - Morphological variations of striped seabream, *Lithognathus mormyrus*, populations along the Tunisian coast. *J. Mar. Biol. Ass. U.K.*, 91: 1261-1271.

HAMMAMI I., BAHRI-SFAR L., KAOUÈCHE M. & BEN HASSINE O.K., 2013. - Morphological characterization of striped seabream (*Lithognathus mormyrus*, Sparidae) in some Mediterranean lagoons. *Cybium,* 37(1-2): 129-141.

HAMZA A., 2003. - Le statut du phytoplancton dans le golfe de Gabès. Thèse de Doctorat, 298 p. Univ. de Sfax, Tunisie.

JØRGENSEN H.B.H., PERTOLDI C., HANSEN M.M., RUZZANTE D.E. & LOESCHCKE V., 2008. - Genetic and environmental correlates of morphological variation in a marine fish: the case of Baltic Sea herring (*Clupea harengus*). *Can. J. Fish. Aquat. Sci*., 65: 389-400.

KAOUÈCHE M., 2012. - Caractérisation parasitologique, morphométrique et génétique des populations tunisiennes de deux espèces de poissons Sparidés : *Diplodus sargus* et *Diplodus vulgaris*. Thèse de Doctorat, 242 p. Fac. Sci. Tunis, Tunisie.

KAOUÈCHE M., BAHRI-SFAR L., GONZALEZ-WANGÜEMERT M., PEREZ-RUZAFA Á. & BEN HASSINE O.K., 2011. - Allozyme and mtDNA variation of white seabream *Diplodus sargus* populations in a transition area between western and eastern Mediterranean basins (Siculo-Tunisian Strait). *Afr. J. Mar. Sci.,* 33(1): 79-90.

LEMOALLE J., 1986. - Étude de la lagune El Bibane. I: Les conditions du milieu. Publication trimestrielle de l'Institut national scientifique et technique d'océanographie et de pêche de Salammbô, Rapport N° 2.

LEMOALLE J. & VIDY G., 1984. - Condition de milieu et pêche dans la lagune hypersaline d'El Biban (Tunisie). *Stud. Rev. CGFM,* 61(1): 175-195.

MACPHERSON E., 1998. - Ontogenetic shifts in habitat use and aggregation in juvenile sparid fishes. *J. Exp. Mar. Biol. Ecol*., 220: 127-150.

MEJRI R., LO BRUTTO S., BEN HASSINE O.K. & ARCULEO M., 2009. - A study on *Pomatoschistus tortonesei* Miller 1968 (Perciformes, Gobiidae) reveals the Siculo-Tunisian Strait (STS) as a breakpoint to gene flow in the Mediterranean basin. *Mol. Phylogenet. Evol.,* 53: 596-601.

MEJRI R., BEN HASSINE O.K. & LO BRUTTO S., 2011. - Genetic architecture of the marbled goby *Pomatoschistus marmoratus* (Perciformes, Gobiidae) in the Mediterranean Sea. *Mol. Phylogenet. Evol*.. 58(2): 395-403.

MEJRI R., LO BRUTTO S., HASSINE N., ARCULEO M. & BEN HASSINE O. K., 2012. -Overlapping patterns of morphometric and genetic differentiation in the Mediterranean goby *Pomatoschistus tortonesei* Miller, 1968 (Perciformes,Gobiidae) in Tunisian lagoons. *Zoology,* 115: 239-244.

MENSCHING H., 1968. - Tunesien. Eine geographische Landeskunde. 281 p. Ort, Verlag & Jahr: Darmstadt, Wissenschaftliche Buchgesellschaft.

MOLINIER R. & PICARD J., 1954. - Éléments de bionomie marine sur les côtes de Tunisie. *Bull. Stn. Océanogr. Pêche Salammbô*, 48: 3-47.

MOUSSA M., BACCAR L. & BEN KHEMIS R., 2005. - La lagune de Ghar El Melh: Diagnostic écologique et perspectives d'aménagement hydraulique. *Rev. Sci. Eau,* 18: 13-26.

NEI M. & CHESSER R.K., 1983. - Estimation of fixation indices and gene diversities. *Ann. Hum. Genet.,* 47: 253-259.

NEI M., 1987. - Molecular evolutionary genetics. Columbia Univ. Press, New York: 39-46.

NEIFAR L., 2001. - Biodiversité et évolution des Plathelminthes parasites des Elasmobranches. Thèse de Doctorat, 326 p. Fac. Sci. Tunis, Tunisie.

OUESLATI A., 1993. - Les côtes de la Tunisie. Géomorphologie et environnement et aptitudes à l'aménagement. Publication Faculté des sciences humaines et sociales de Tunis, Géographie, ser. 2, 39: 1-500.

PALMA J. & ANDRADE J.P., 2002. - Morphological study of *Diplodus sargus, Diplodus puntazzo*, and *Lithognathus mormyrus* (Sparidae) in the Eastern Atlantic and Mediterranean Sea. *Fish. Res.,* 57: 1-8.

PALUMBI S.R., 1994. - Genetic divergence, reproductive isolation, and marine speciation. *Annu. Rev. Ecol. Syst*., 25: 547-572.

PASTEUR N., PASTEUR G., BONHOMME F., CATALAN J. & BEITTON-DAVIDIAN J., 1987. - Manuel technique de génétique par électrophorèse des Protéines. 217 p. Paris: Editions Lavoisier.

PATARNELLO T., VOLCKAERT F.A.M.J. & CASTILHO R., 2007. - Pillars of Hercules: is the Atlantic-Mediterranean transition a phylogeographical break? *Mol. Ecol.,* 16: 4426-4444.

PEPIN P. & CARR S.M., 1993. - Morphological, meristic and genetic analysis of stock structure in juvenile Atlantic Cod *(Gadus morhua)* from the Newfoundland shelf. *Sci. Counc. Meet.,* 31: 1-12.

PERGENT G. & KEMPF M., 1993. - L'environnement marin côtier en Tunisie. Rapport IFREMER DEL Brest 92.06, 3: 1-539.

REIST J.D., 1985. - An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. *Can. J. Zool.,* 63: 1429-1439.

ROSECCHI E., 1985. - L'alimentation de *Diplodus annularis, Diplodus sargus, Diplodus vulgaris* et *Sparus aurata* (Pisces, Sparidae) dans le golfe du Lion et les lagunes littorales**.** *Rev. Trav. Inst. Pêches Mar.*, 49(3-4): 125-141.

SARÀ M., FAVAROLO E. & MAZZALO A., 1999. - Comparative morphometrics of sharp-snout seabream (*Diplodus spuntazzo* Cetti, 1777) reared in different conditions. *Aquacult. Eng.,* 19: 195-209.

SHAKLEE J.B., ALLENDORF F., MORIZOT D.C. & WHITT G.S., 1990. - Gene nomenclature for protein-coding loci in fish. *Trans. Am. Fish. Soc.,* 119: 2-15.

STEFANNI S. & THORLEY J.H., 2003. - Mitochondrial DNA phylogeography reveals the existence of an Evolutionarily Significant Unit of the sand goby *Pomatoschistus minutus* in the Adriatic (Eastern Mediterranean). *Mol. Phylogenet. Evol.,* 28: 601-609.

STRAUSS R.E. & BOOKSTEIN F.L., 1982. - The truss: body from reconstructions in morphometrics. *Syst. Zool.,* 31: 113-135.

STEARNS S.C., 1983. - A natural experiment in life-history evolution: field data on the introduction of mosquitofish (*Gambusia affinis*) to Hawaii. *Evolution,* 37: 601-617.

TEKAYA N., 2009. - Comportement trophique des espèces du genre *Diplodus* de la côte Est de la Tunisie. Mastère, 195 p. Fac. Sci. Tunis, Tunisie.

TURAN C., 2004. - Stock identification of Mediterranean horse mackerel (*Trachurus mediterraneus*) using morphometric and meristic characters. *ICES J. Mar. Sci.,* 61: 774-781.

TURAN C. & BASUSTA N., 2001. - Comparison of morphometric characters of twaite shad (*Alosa fallax nilotica*, Geoffroy Saint-Hilaire, 1808) among three areas in Turkish seas. *Bull. Fr. Pêche Piscic.,* 362/363: 1027-1035.

TURAN C., ERGÜDEN D., TURAN F. & GÜRLEK M., 2004. - Genetic and morphologic structure of *Liza abu* (Heckel, 1843). Populations from the rivers Orontes, Euphrates and Tigris. *Turk. J. Vet. Anim. Sci.,* 28: 729-734.

VIA S., GOMULKIEWICZ R., DE JONG G., SCHEINER S.M., SCHLICHTING C. D. & VAN TIENDEREN P.H., 1995. - Adaptive phenotypic plasticity: consensus and controversy. *Trends Ecol. Evol.,* 10: 212-217.

WAINWRIGHT P.C, BELLWOOD D.R., WESTNEAT M.W., GRUBICH J.R. & HOEY A.S., 2004. - A functional morphospace for the skull of labrid fishes: patterns of diversity in a complex biomechanical system. *Biol. J. Linn. Soc. Lond.,* 82: 1-25.

WALLS S.C., BELANGER S.S., & BLAUSTEIN A.R., 1993. - Morphological variation in a larval salamander: dietary induction of plasticity in head shape. *Oecologia*, 96: 162-168.

WEIR B.S. & COCKERHAM C., 1984. - Estimating *F*-statistics for the analysis of population structure. *Evolution,* 38: 1350-1358.

WRIGHT S., 1969. - Evolution and genetics of populations (Vol. 2). The theory of gene frequencies. Chicago: Univ. of Chicago.

ZAKHAMA R., SGHAIER Y.R. & CHARFI F., 2005. - Biodiversité des amphipodes Gammaridae associés à l'herbier de *Posidonia oceanica* des côtes orientales de la Tunisie. *INOC- Mar. Coast. Protected Areas*, 274-277.

ZARDOYA R., CASTILHO R., GRANDE C., FAVRE-KREY L., CAETANO S., MARCATO S., KREY G. & PATARNELLO T., 2004. - Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*) in the Mediterranean Sea. *Mol. Ecol.,* 13: 1785-1798.

ZITARI-CHATTI R., CHATTI N., FULGIONE D., CAIAZZA I., APREA G., ELOUAER A., SAID K. & CAPRIGLIONE T., 2009. - Mitochondrial DNA variation in the caramote prawn *Penaeus (Melicertus) kerathurus* across a transition zone in the Mediterranean Sea. *Genetica,* 136: 439-447.

Annex I. - Loadings from discriminant function analysis. Univariate analysis (ANOVA) for all measurements. Significance levels: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. %: discrimination percentage of each function. In bold: more discriminant variables along the two axes.

	F	DF1	DF2
		28%	24%
V1: X1_2	30.735^{***}	-0.062	**0.633**
V2: X2_3	62.492^{***}	**-0.869**	-0.242
V3: X3_4	3.815^{***}	-0.198	-0.073
V4: X4_5	20.485^{***}	-0.337	0.4154
V5: X5_7	2.938^{***}	-0.103	-0.055
V6: X7_6	5.832^{***}	0.043	-0.159
V7: X6_8	5.594^{***}	-0.204	0.156
V8: X8_9	13.656^{***}	-0.471	0.320
V9: X9_10	8.915^{***}	-0.395	-0.241
V10: 10_11	9.957^{***}	-0.258	0.421
V11: X11_1	13.242^{***}	-0.504	-0.016
V12: X2_11	31.113^{***}	-0.208	**0.577**
V13: X11_3	38.713^{***}	-0.589	0.401
V14: X3_10	26.096^{***}	-0.452	0.302
V15: X10_4	8.166^{***}	-0.311	-0.241
V16: X4_8	2.504^{***}	0.099	-0.025
V17: X8_5	1.291	0.024	-0.056
V18: X5_6	9.340^{***}	0.047	-0.359
V19: X6_4	3.207^{***}	0.004	0.097
V20: X4_9	4.279^{***}	0.084	0.043
V21: X9_3	9.042^{***}	-0.034	-0.027
V22: X5_9	3.861^{***}	-0.022	0.220
V23: X10_2	5.139^{***}	-0.219	0.214
V24: X1_10	26.583^{***}	-0.399	0.349
V25: X1_3	38.803^{***}	**-0.642**	0.363
V26: X1_12	17.997^{***}	0.011	0.475
V27: X1_15	16.636^{***}	-0.493	0.272
V28: X2_15	17.316^{***}	-0.371	0.484
V29: 11_15	27.932^{***}	-0.357	**0.506**
V30: X13_14	24.893^{***}	**-0.647**	0.242
V31: X16_17	22.901^{***}	-0.542	0.276